

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application. Inserted text is indicated with underlining and deleted text is indicated with ~~striketrough~~. Claim status is indicated as **currently amended**, **original** or **cancelled**. These claim amendments and the new claims do not introduce new matter.

Listing of the Claims:

1. **(original)** A composition comprising an orthogonal aminoacyl-tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates an O-tRNA with a redox active amino acid.
2. **(original)** The composition of claim 1, wherein the O-RS comprises an amino acid sequence comprising SEQ ID NO.: 1, or a conservative variation thereof.
3. **(original)** The composition of claim 1, wherein the O-RS preferentially aminoacylates the O-tRNA with an efficiency of at least 50% of the efficiency of a polypeptide comprising an amino acid sequence of SEQ ID NO.: 1.
4. **(original)** The composition of claim 1, wherein the O-RS is derived from a *Methanococcus jannaschii*.
5. **(original)** The composition of claim 1, comprising a cell.
6. **(original)** The composition of claim 5, wherein the cell is an E. coli cell.
7. **(original)** The composition of claim 1, comprising a translation system.
8. **(original)** A cell comprising a translation system, wherein the translation system comprises:
 - an orthogonal -tRNA (O-tRNA);
 - an orthogonal aminoacyl-tRNA synthetase (O-RS); and,
 - a redox active amino acid;
 - wherein the O-tRNA recognizes a first selector codon, and the O-RS preferentially aminoacylates the O-tRNA with the first redox active amino acid.

9. (original) The cell of claim 8, wherein the O-RS preferentially aminoacylates the O-tRNA with an efficiency of at least 50% of the efficiency of a polypeptide comprising an amino acid sequence of SEQ ID NO.: 1.
10. (original) The cell of claim 8, wherein the O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in SEQ ID NO.: 2, or a complementary polynucleotide sequence thereof, and wherein the O-RS comprises an amino acid sequence comprising SEQ ID NO.: 1, or a conservative variation thereof.
11. (original) The cell of claim 8, wherein the cell further comprises an additional different O-tRNA/O-RS pair and unnatural amino acid, wherein the O-tRNA recognizes a second selector codon and the O-RS preferentially aminoacylates the O-tRNA with the second unnatural amino acid.
12. (original) The cell of claim 8, wherein the cell is a non-eukaryotic cell.
13. (original) The cell of claim 12, wherein the non-eukaryotic cell is an *E. coli* cell.
14. (original) The cell of claim 8, further comprising a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises a selector codon that is recognized by the O-tRNA.
15. (original) An *E. coli* cell, comprising:
 - an orthogonal tRNA (O-tRNA);
 - an orthogonal aminoacyl- tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates the O-tRNA with a redox active amino acid;
 - the redox active amino acid; and,
 - a nucleic acid that encodes a polypeptide of interest, wherein the nucleic acid comprises the selector codon that is recognized by the O-tRNA.
16. (original) The *E. coli* cell of claim 15, wherein the O-RS preferentially aminoacylates the O-tRNA with an efficiency of at least 50% of the efficiency of a polypeptide comprising an amino acid sequence of SEQ ID NO.: 1.
17. (original) An artificial polypeptide comprising SEQ ID NO. 1.
18. (original) An artificial polynucleotide that encodes a polypeptide of claim 17.

19. **(original)** A vector comprising or encoding a polynucleotide of claim 18.
20. **(original)** The vector of claim 19, wherein the vector comprises a plasmid, a cosmid, a phage, or a virus.
21. **(original)** The vector of claim 19, wherein the vector is an expression vector.
22. **(original)** A cell comprising the vector of claim 19.
23. **(original)** A method for identifying an orthogonal-aminoacyl-tRNA synthetase for use with an O-tRNA that utilizes a redox amino acid, the method comprising:
subjecting to selection a population of cells of a first species, wherein the cells each comprise:
 - 1) a member of a plurality of aminoacyl-tRNA synthetases (RSs);
 - 2) the orthogonal tRNA (O-tRNA) derived from one or more species; and,
 - 3) a polynucleotide that encodes a selection marker and comprises at least one selector codon;wherein cells that are enhanced in suppression efficiency as compared to cells lacking or comprising a reduced amount of the member of the plurality of RSs that comprises an active RS that aminoacylates the O-tRNA; and,
selecting the active RS that aminoacylates the O-tRNA with the redox active amino acid, thereby providing the orthogonal -aminoacyl-tRNA synthetase for use with the O-tRNA.
24. **(original)** The method of claim 23, wherein the selection comprises a positive selection and the selection marker comprises a positive selection marker.
25. **(original)** The method of claim 23, wherein the plurality of RSs comprise mutant RSs, RSs derived from one or more species other than the first species or both mutant RSs and RSs derived from a species other than the first species.
26. **(original)** An orthogonal aminoacyl-tRNA synthetase identified by the method of claim 23.
27. **(currently amended)** A method of producing a protein in a cell with a redox active amino acid at a specified position, the method comprising:

a) growing, in an appropriate medium, the cell, where the cell comprises a nucleic acid that comprises at least one selector codon and encodes a protein; and,

b) providing the redox active amino acid;

wherein the cell further comprises:

an orthogonal -tRNA (O-tRNA) that functions in the cell and recognizes the selector codon; and,

an orthogonal aminoacyl-tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the redox active amino acid, wherein said O-RS:

i) has an efficiency that is at least 50% of the efficiency observed for a translation

system comprising said O-tRNA, said redox active amino acid, and an

aminoacyl-tRNA synthetase comprising the amino acid sequence of SEQ ID

NO: 1; or

ii) preferentially aminoacylates said O-tRNA with a redox active amino acid

selected from the group consisting of a 3,4-dihydroxy-L-phenylalanine (DHP), a

3,4,5-trihydroxy-L-phenylalanine, a 3-nitro-tyrosine, a 4-nitro-phenylalanine

and a 3-thiol-tyrosine; and

c) incorporating the redox active amino acid into the specified position in the protein during translation of the nucleic acid with the at least one selector codon, thereby producing the protein with a redox active amino acid at a specified position.

28. (currently amended) The method of claim 27, wherein the O-RS comprises an a amino acid sequence which comprises SEQ ID NO.: 1.

29. (original) The method of claim 27, wherein the cell is a non-eukaryotic cell.

30. (original) The method of claim 29, wherein the non-eukaryotic cell is an *E. coli* cell.

31. (currently amended) A composition comprising a protein, wherein the protein comprises a redox active amino acid selected from the group consisting of: a 3,4-dihydroxy-L-phenylalanine (DHP), a 3,4,5-trihydroxy-L-phenylalanine, a 3-nitro-tyrosine, a 4-nitro-phenylalanine, and a 3-thiol-tyrosine.

32. (cancelled)

33. (original) The composition of claim 31, wherein the protein comprises an amino acid sequence that is at least 75% identical to that of a wild-type therapeutic protein, a diagnostic protein, an industrial enzyme, or portion thereof.
34. (original) The composition of claim 31, wherein the composition comprises a pharmaceutically acceptable carrier.
35. (new) The composition of claim 31, wherein the protein comprises two or more unnatural amino acids.
36. (new) The composition of claim 31, wherein the protein comprises two or more unnatural amino acids, wherein two or more of said unnatural amino acids are the same.
37. (new) The composition of claim 31, wherein the protein comprises two or more redox active amino acids.
38. (new) The composition of claim 31, wherein the protein comprises two or more unnatural amino acids that are different.
39. (new) The composition of claim 31, wherein the protein comprises a myoglobin polypeptide or a portion thereof.
40. (new) The composition of claim 39, wherein said myoglobin polypeptide comprises a substitution with at least one redox active amino acid.
41. (new) The composition of claim 31, wherein the protein is capable of undergoing oxidation.
42. (new) The composition of claim 31, wherein the protein is capable of undergoing oxidation or improved oxidation, and wherein said oxidation or improved oxidation requires said redox active amino acid.
43. (new) The composition of claim 31, wherein the redox active amino acid is 3,4-dihydroxy-L-phenylalanine (DHP).
44. (new) The composition of claim 31, wherein the redox active amino acid is a redox catalyst.